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Sperm dumping in *Centrobolus inscriptus* Attems (Spirobolida: Trigonulidae)

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Abstract

Differential ejaculate usage was tested in the diplopod *Centrobolus inscriptus*. Copulation duration, post-mating interval, and ejaculate volume (disintegrations per minute of H³⁺) were analysed for single and double mating sequences. During the 24h post-mating, ejaculate volumes decline was identified. The significant drop in ejaculate volumes (dpm) post-mating ($Z = 3.00$, $n = 6, 7$, $P = 0.003$) indicates a female process thought to be sperm dumping.

Keywords: *Centrobolus*, copulation, diplopod, female, mating

1. Introduction

Sperm dumping has been studied as examples of cryptic female choice in spiders [1] *Physocyclus globosus* [2], armored goblin spiders [3-4], Palearctic gift-giving spider *Pisaura mirabilis* [5], *Orsolobus pucara* [6], fly *Drosophila simulans* [7-8], millipede *Alloporus uncinatus* [9], Odonata [10], *Apis* bees [11], moth *Ephestia kuehniella* [12], water strider *Gerris lateralis* [13]. The active release of ejaculate post-insemination and copulation duration was studied in *Centrobolus inscriptus* where sperm or ejaculate precedence correlates with prolonged second copulation duration and re-analysis of sperm volumes from single and double mating experiments with 24 hour (h) delays post-mating [14-26]. In order to provide supporting evidence for female control of ejaculate and sperm dumping after mating, ejaculate volumes released from females 24h after mating was studied and compared alongside double matings.

2. Materials and methods

Chersastus inscriptus were collected from Twin Streams (28°55'S, 31 °45'E) (1995 - 1998). Live specimens of each sex were taken to a laboratory, kept at 25 °C; 70% relative humidity; 12:12 h light-dark. Vegetables were provided *ad lib*. Unisex groups were kept in plastic containers lined by moist vermiculite (± 5 cm deep). Ejaculate volume was measured as disintegrations per minute (dpm) tritium in a 1600 scintillation counter (low count reject = 0; dpm multiplier = 1). Millipedes were marked on posterior segments with colored tipex and placed in glass aquaria (30 X 22 X 22 mm). Pairs were isolated in plastic beakers (13cm diameter) and mating timed. Double mating sequences were performed to test for order or interval effect. Mating order was controlled by mating females with labelled and un-labelled males in reciprocity. Females were given the opportunity for second mating immediately (0-h delay) or later (24-h delay). As controls, females that had single mating with labelled males were dissected immediately or 24 h later. Females that had single mating with un-labeled males were dissected to control for background radiation. Data were analyzed with Pearson's correlation (r), t-tests (t), Spearman's rank order correlation coefficients (r), two-tailed Mann-Whitney U-tests (Z), and Wilcoxon tests (T). A Kruskal Wallis 1-way ANOVA (H) was used to test for differences in dpm data prior to Mann-Whitney U-tests.

3. Results

I successfully demonstrate differential ejaculate usage or control by female millipedes post-copulation. There was a significant drop in ejaculate volumes (dpm) during 24h after single mating (Figure 1: $Z = 3.00$, $n = 6, 7$, $P = 0.003$).

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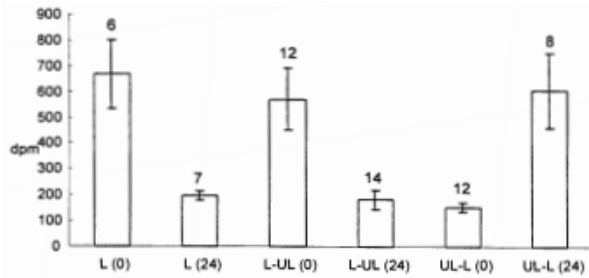


Fig 1: Ejaculate volumes (mean disintegrations H^{3+} per minute \pm SE) obtained from the spermathecae of *Centrobolus inscriptus* females engaged in single and double mating experiments where (0) and (24) represent the period (hours) before dissection and the remating intervals, respectively. L - H^{3+} labelled male, UL – unlabelled male.

4. Discussion

Here differential ejaculate usage is acknowledged in the millipede *C. inscriptus*. It is known that the re-mating interval negatively relates to second duration ($y = 36 + 0.01x$) and relative ejaculate volume increased from 21% to 77% in favour of second males after 24h intervals^[14]. This is a male process and as yet little or no supporting evidence for a female process are known in millipedes with one exception in the spirostreptidan millipede^[9].

Evidence is shown for ejaculate dumping as a form of cryptic female control by the sudden drop in ejaculate volumes 24 post-mating. This may contribute toward understanding the ecology of sperm^[27].

5. Conclusion

During the 24h post-mating ejaculate volumes in *C. inscriptus* consistently decline and this is due to sperm dumping.

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